Table 4
HABITATS OF BACTERIOPHAGES[a]

EXHIBIT A

Water	Surface water:	lakes, ponds, streams, rivers, cisterns, drinking water, fish farm effluents, trout hatcheries, hot springs, irrigation channels, paddy fields, salt ponds, lagoons
	Seawater:	brack water, coastal, deep sea, sediments, tidal ponds
	Sewage:	raw or chlorinated, activated sludge, reactor effluents, from slaughterhouses
Soil	Types:	arable, clay, compost, forest, moor, mud and silt, podzol, rhizosphere, sand
	From:	alfalfa, soybean, and vegetable fields; barnyards, cow sheds, chicken houses, gardens, greenhouses, lawns
Air	Droplets, dust particles	
Plants	Organs:	buds, leaves, nodules (leguminous plants), rotting fruits, roots, seeds, stems and straw; crown gall tumors
	Species:	healthy or diseased alfalfa, barley, beans, broccoli, Brussels sprouts, buckwheat, clover, cotton, cucumber, oats, peas, peach trees, radish, rutabaga, rye, timothy, tobacco, tomatoes, wheat.
	Miscellany:	grass clippings, moldy silage
Animals	Body fluids and excreta:	blood, cerebrospinal fluid, feces, nasopharyngeal secretions, pus, saliva, sputum, urine
	Organs:	cecum, gizzard, intestine, nostrils, pharynx, placenta, rumen, tonsils, vagina
	Species:	healthy or diseased man, ass, bee, cattle, chicken, clam, crab, *Culex pipiens*, earthworm, ground squirrel, guinea pig, horse, housefly, mouse, oyster, pidgeon, pig, rabbit, rat, shrimp, turkey, water buffalo, *Xenopsylla cheopis*; various fishes, mosquitoes, and mussels; 9 species of zoo birds and 33 species of zoo mammals[b]
Food	Dairy products:	raw and skim milk, butter, butter milk, cheeses (Cheddar, cottage, Swiss), cheese starters (Bel Paese, Emmenthal, Gorgouzola, Mozzarella), cheese wheys, yogurt and yogurt starters
	Meat and fish:	chicken, ground beef, meat starters, salami, steaks; fish fillets, fish sauce
	Other:	lactic acid beverage, oysters, sake starter, spoiled cabbage, wine
Miscellany	Culture broth of fermentors; fetal serum of calf, lamb, and rabbit	

[a] For references prior to 1965, see Raettig.[67,68]
[b] Reference 80.

the other hand, the bacterial host is always known, and the phage may be isolated and identified. By contrast, the electron microscopical approach allows study of complete phage populations, but their identity can only be guessed. An intermediate approach would be enrichment followed by sedimentation and electron microscopical examination of washed sediments.[66] Although not quantitative, this gives satisfactory pictures and allows reasonably certain identification of the more frequent phage types.

The best-studied habitat is water, especially sewage; in addition, much work has been done on phages in the dairy and fermentation industry. Most studies are not environmental at all, but are aimed at the isolation of specific phages. Others have specific subjects, e.g., investigation of phages of lactic streptococci in cheese factories, the role of phages in animal and plant diseases, phages as models for ecological studies of animal viruses, or phages as indicators of fecal pollution. The phages so studied are often poorly identified or not at all. In the case of "coliphages", for example, it is seldom known whether they are tailed, cubic, or filamentous, and changes are that they lyse not only *E. coli*, but a host of other enterobacteria as well. These studies have produced numerous data on phage resistance against sunlight, oxygen, chemicals, and other agents, which may be found elsewhere.[69] However, they contain relatively little information for comparative virology and are thus outside the scope of this book.

Table 5
FREQUENCY OF PHAGES IN SELECTED ENVIRONMENTS

Sample	Technique	Phages counted	PFU[a]	Ref.
Water				
Sewage, activated sludge	EM[b]	Total	1.3×10^6—9.5×10^7	70,71
Seawater	EM	Total	Over 10^3—10^4	73
Sewage	Plaque assay	Coliphages	10—10^7	76-81
	Plaque assay	RNA coliphages	40—5.1×10^3	82
River water, sediments	Plaque assay	Coliphages	0—2.5×10^4	83
River water	Plaque assay	Coliphages	0.2—5.3×10^3	84
Sewage	Plaque assay	Cyanophages	0—9.9×10^8	85
Soil	Plaque assay	Actinophages	Up to 2.3×10^4/g	86
Feces				
Human	Plaque assay	Coliphages	0—10^9/g	87
Mammals and birds	Plaque assay	Coliphages	10—10^7/g	80
Rumen				
Cattle	EM	Total	5×10^7	72
Cattle and sheep	EM	Total	Over 10^9	74
Oysters, clams	Plaque assay	*Vibrio*	Up to 10^6/g	88

[a] PFU, plaque-forming units; per milliliter unless otherwise specified.
[b] EM, electron microscopy.

B. Frequency and Variety of Phages in Nature

In a general way, data on total phage populations in nature are fragmentary. Phages are counted by plating and electron microscopy. Plaque assays cannot indicate total phage numbers in heterogeneous populations[70] and can hardly be compared from one laboratory to another. Furthermore, phages may adsorb to organic and inorganic matter and escape detection. In some cases, however, plaque assays have revealed high phage numbers which are comparable to those obtained by electron microscopy (Table 5). On the other hand, some phages are very rare and can only be detected after concentration. A combination of the plaque assay with an adsorption-elution technique was needed to reveal the presence of one *Acinetobacter* phage per 5 mℓ of river water.[65]

Electron microscopical phage counts have only been done on water and rumen. Methods include (1) concentration of phages in a particle sedimentation rotor and examination of pseudoreplicas,[70-72] (2) concentration of phages by filtration,[73] and (3) the spray droplet technique.[74] The first two procedures use uranyl acetate for staining. Unfortunately, this stain tends to cause positive staining, making phage particles difficult or impossible to identify (Chapter 6, this volume); in particular, the pseudoreplica technique results in poor pictures. Replacement of uranyl acetate by phosphotungstate would probably be helpful. The spray droplet technique uses phosphotungstate, allows good phage identification, and seems to be preferable for phage-rich environments. Electron microscopy is inappropriate for detection of rare phages. Table 5 includes data on phage frequencies from recent literature. It may be noted that all coliphage counts by plaque assay are from the same laboratory.

As revealed by electron microscopy, phage populations in nature may be extremely varied (Figure 2). More than 40 different phage-like entities were detected in cattle and sheep rumen,[74] turkey ceca contain 4 to 10 phage varieties per bird,[75] and sediments from enriched sewage contain up to 10 different particles.[66] Moreover, electron microscopy allows detection of phages for bacteria that are difficult or impossible to grow, e.g., *Selenomonas*.[18] Unfortunately, electron microscopy has so far rarely been applied to phage ecology and has certainly not been used to its full potential.

http://en.wikipedia.org/wiki/Phage_ecology#Vastness_of_phage_ecology

Phage ecology

From Wikipedia, the free encyclopedia

[This is an excerpt from the web page]

Bacteriophages (phages), potentially the most numerous "organisms" on Earth, are the viruses of bacteria (more generally, of prokaryotes[1]). **Phage ecology** is the study of the interaction of bacteriophages with their environments.[2] Phage ecology is increasingly an important component of sessions and symposiums associated with phage meetings as well as general microbiological meetings.

Contents

[hide]

[edit] Introduction to phage ecology

[edit] Vastness of phage ecology

Phages are obligate intracellular parasites meaning that they are able to reproduce only while infecting bacteria. Phages therefore are found only within environments that contain bacteria. Most environments contain bacteria, including our own bodies (there called normal flora). Often these bacteria are found in large numbers. As a consequence, phages are found almost everywhere.

As a rule of thumb, many phage biologists expect that phage population densities will exceed bacterial densities by a ratio of 10-to-1 or more (VBR or virus-to-bacterium ratio; see [1] for a summary of actual data). As there exist estimates of bacterial numbers on Earth of approximately 10^{30}[2], there consequently is an expectation that 10^{31} or more individual virus (mostly phage[3]) particles exist[4], making phages the most numerous category of "organisms" on our planet.

Bacteria (along with archaeabacteria) appear to be highly diverse and there possibly are millions of species[5]. Phage-ecological interactions therefore are quantitatively vast: huge numbers of interactions. Phage-ecological interactions are also qualitatively diverse: There are huge numbers of environment types, bacterial-host types[6], and also individual phage types[7]).

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EXHIBIT C

Virioplankton: Viruses in Aquatic Ecosystems†

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INTRODUCTION

From studies of the genetics and biology of viruses has come a more profound understanding of the basic biological processes of life, not the least of which has been the discovery of DNA as the carrier molecule of genetic information (125) and mRNA as an intermediate molecule in the transfer of genetic information to the ribosomes (43). Other breakthroughs in molecular biology attributable to bacteriophage models are the definition and mapping of the first gene (18); discovery of the discontinuous nature of DNA replication (222); discovery of restriction endonucleases (212); and the mechanics of gene regulation (261). Indeed, basic research on the biology of the bacteriophage has been fundamental to the establishment of the field of molecular biology (73). The value of basic research to technological and economic advancement is perhaps best illustrated by the historical link between basic bacteriophage biology and the present-day, multibillion dollar biotechnology industry.

In contrast to extensive information on the biology and genetics of viruses, there is only a limited understanding of the

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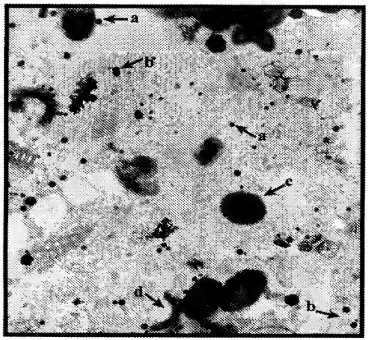

FIG. 1. Transmission electron micrograph of an unfiltered Chesapeake Bay water sample (magnification, ca. ×36,000). a, short-tailed or nontailed virus-like particle; b, tailed virus-like particle; c, bacterium, coccal morphotype; d, bacterium, vibrio morphotype.

occurrence and distribution of viruses in microbial ecosystems and in situ relationships between viral and host communities in the natural environment. The lack of recognition of viruses as naturally occurring organisms was most notably exposed with the discovery that viruses are abundant in a variety of aquatic environments, often exceeding bacterial abundance by an order of magnitude (19, 257). It was a surprise to learn from direct transmission electron microscope examination of marine virio- and bacterioplankton the astounding abundance of virus-like particles in seawater, considering that marine bacteriophages were first described in detail more than 35 years ago (301, 302, 331, 332). As an example, a transmission electron micrograph of unfiltered Chesapeake Bay water is shown in Fig. 1. The realization that in most aquatic environments the virioplankton is the most abundant plankton class has revived scientific investigation into the natural state of viruses in aquatic environments. Important questions raised by discovery of the abundance of viruses in natural ecosystems challenge accepted views of the aquatic microbial food web and the hypothesized singular role of obligate parasites in controlling microorganism population abundance and diversity.

SCOPE OF THE REVIEW

This review is divided into four sections. The first and second sections cover methods for viral direct counting, reports of naturally occurring virioplankton abundance in a variety of aquatic environments, and the correlation between changes in virus abundance and changes in other important ecological parameters. The third examines aspects of aquatic bacteriophage biology which have a significant influence on host infection in aquatic environments. The final section focuses on viral infection and lysis, as both a factor in the mortality of host populations and a mechanism influencing genetic and clonal diversity of host populations. In general, the discussion is focused on in situ measurement of virioplankton populations. Model phage-host systems are included, as appropriate, for background information. Readers interested in aquatic phage-host relationships will find relevant information in reviews by Børsheim (29) and Proctor (253) and an earlier review by Moebus (193). A brief synopsis of current views on marine virus ecology was recently provided by Furhman (91). The distribution and survival of human and animal pathogenic enteroviruses in aquatic environments are not covered herein, since detection and distribution of human disease-causing viruses in natural waters have been extensively reviewed elsewhere (99, 103, 104, 112, 182).

ENUMERATION OF VIRUSES IN WATER SAMPLES

Introduction

Discovery of the abundance of viruses in natural waters reflects the development of direct counting methods for bacterial enumeration. From the use of direct-counting methods to enumerate bacteria in environmental samples (127, 381), it has been found that viable counts, obtained using culture methods, significantly underestimate the number of bacteria in the sample. This finding is not surprising since it was suspected from the early days of bacteriology, when staining methods were used to enumerate bacteria. Newer techniques yielded results that led many investigators to reject conclusions regarding the ecology of bacteria obtained solely by culture. Molecular methods developed for analyzing bacterial population dynamics and diversity have revealed large populations of unculturable bacteria in the environment, further fueling speculation that bacterial diversity may be 100 to 1,000 times greater than that suggested by results of studies involving culture methods (60).

Indirect, Viable Counting of Bacteriophage and Viruses in Water Samples

Indirect titer determination by plaque assay (4), coupled with the most-probable-number method (150, 309), has routinely been used to enumerate viruses in water samples but has only recently been used to elucidate the ecology of viruses. For example, the abundance and distribution of coliphages in natural water samples have been determined by a plaque assay (99, 118, 244). In general, estimates of the abundance of specific viruses by culture methods have been so low that preconcentration of viruses was necessary prior to inoculation and enumeration (titers per liter). The distribution and abundance of phages infecting autochthonous bacterial hosts in a natural body of water, such as the Chesapeake Bay, illustrate the difficulty in interpreting data on the abundance of specific bacteriophages by culture assay (K. E. Wommack, R. T. Hill, J. Ravel, and R. R. Colwell, Abstr. 96th Gen. Meet. Am. Soc. Microbiol. 1996, abstr. N-23, p. 159, 1996). For example, 36 water samples collected at six stations during the year yielded only 10 samples that were positive for bacteriophages infecting one or more of the indicator strains. Of the 10 successful bacteriophage isolations from Chesapeake Bay water samples, only two of the titers exceeded the detection limit of 1 PFU. After taking into account the 10- to 100-fold concentration of virioplankton within the water samples, 7 PFU liter^{-1} was the final abundance estimate. However, direct microscopic examination revealed 100- to 1,000-fold more virus particles in each water sample.

Even though culture-based methods are not efficient in the